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Award Number: W81XWH-08-1-0148

TITLE: Diagnostic and Therapeutic Cancer Care Equipment

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CONTRACTING ORGANIZATION: Boston Medical Center Corporation
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REPORT DATE: July 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-07-2009	Annual	01 Jul 2008-30 Jun 2009

4. TITLE AND SUBTITLE
Diagnostic and Therapeutic Cancer Care Equipment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0148

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Boston University Medical Center Corporation

Boston, MA 02118-2906

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND

FORT DETRICK, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Acute lung injury (ALI) is a complex condition associated with diffuse injury to the alveolar epithelial gas exchange surface, resulting in marked impairment in the ability to oxygenate blood. The goal of our application is to develop strategies to treat the ALI syndromes that complicate cancer care. To reiterate, accumulated data show that ALI is associated with severe infections, exposure to toxins, trauma, and multiple blood transfusions. Cancer patients are vulnerable to development of ALI as a result of the immunosuppressive effects of chemotherapy and the debilitating effects of cancer on overall well-being. Of note, military personnel are also at risk for development of ALI because of battle induced trauma and the consequent need for blood transfusions. In Project 1, we propose to perform a clinical study that will test the whether so-called variable ventilation is superior to conventional mechanical ventilation in patients with ALI. In the preclinical Project 2 study in mice, we proposed to identify a progenitor cell population that could be delivered to augment epithelial reconstitution of an injured alveolar gas exchange surface. In the past year we have: 1) successfully obtained IRB approval and are in the process of submitting an IND application to begin a Phase I study of variable ventilation, 2) developed appropriate software for the clinical study, and 3) begun to develop sources of cell progenitor populations that can be utilized for epithelial reconstitution.

15. SUBJECT TERMS
Lung injury, cancer, ventilator, stem cells

16. SECURITY CLASSIFICATION OF: unclassified			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
			UU		USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U		16	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Introduction

The goal of our application is to develop strategies to treat the acute lung injury syndromes that complicate cancer care (ALI). To reiterate, accumulated data show that ALI is associated with severe infections, exposure to toxins, trauma, and multiple blood transfusions. Cancer patients are particularly vulnerable to development of ALI as a result of the immunosuppressive effects of chemotherapy and the debilitating consequences of cancer on overall well-being. Further, patients with cancer receive chemotherapeutic agents, which themselves can cause diffuse lung injury. As discussed in the parent grant, ALI is complex conditions associated with diffuse injury to the alveolar epithelial gas exchange surface, resulting in marked impairment in the ability to oxygenate blood. Of note, military personnel are also at risk for development of ALI because of battle induced trauma and the consequent need for blood transfusions. To meet our goal, this grant had 2 Projects. In Project 1, we proposed to build upon findings from animal studies to determine the optimal method for mechanical ventilation of patients with ALI. The critical need for better modes of ventilation derives from collected observations demonstrating that current ventilatory modalities may actually worsen underlying ALI. Specifically, we proposed to evaluate the efficacy of so-called variable ventilation in patients with ALI relative to conventional ventilation. In Project 2 of this proposal, we are focused on identifying progenitor cell populations that could be delivered to patients with ALI to augment epithelial reconstitution of the alveolar gas exchange region. This is a pre-clinical study that uses laboratory mice, mouse cells, and a well-described model of chemotherapy induced ALI. In this report, I will discuss the progress made in these 2 Projects.

Body of Progress Report

Below is a summary of the progress and achievements for the 2 Projects that comprised the original parent proposal.

Project 1: We will determine if variable ventilation is a more effective mode of ventilation in patients with ALI.

During year one of this Project, we have made significant progress towards initiating a Phase I study of the Variable Ventilation mode in humans with acute lung injury. We have hired a Study Coordinator who has been preparing the detailed regulatory paperwork. One important achievement is that we have initiated a variety of educational programs to ensure that clinical personnel (nursing, respiratory therapy, physicians) are informed about the variable ventilation study. This is absolutely necessary for patient safety and to ensure that the data generated will be clearly interpretable.

Most importantly, after a rigorous review the study protocol for this Project has been approved by the Boston University School of Medicine Institutional Review Board (approval letter attached, in appendix). Specifically, we have approval to begin this Phase I clinical trial after receipt of an FDA Investigational Device Exemption (IDE). Though we realize that this may be a lengthy process, we are hopeful that this will occur relatively smoothly and in a timely manner. Indeed, the FDA IDE process is underway. We have completed the Pre-IDE review (see Pre-IDE letter attached) and have prepared a final IDE application that is ready for submission pending completion of several factors involving ventilator engineering.

One major element of variable ventilation is the necessary programming of ventilators for safe and reliable delivery of this ventilatory mode. Most notably, we have succeeded in developing the necessary variable ventilation software. This was achieved in the laboratory of Dr. Bela Suki. In view of this, we are now involved in engineering work that focuses on assuring reliable communication between the laptop computer running the variable ventilation program and the ventilator. After achieving dependable communication, the variable ventilation mode will be performance-tested in an in vitro model, and a final FDA IDE report will be submitted. We anticipate that these steps will be completed by 9/1/2009. If all goes according to plan, we should begin enrolling patients for this Phase I study in the fall of 2009.

As discussed in the parent grant, this is a short-term feasibility, safety, and efficacy study. Our long term goal is to have sufficient data to support the planning and execution of a large scale study that can test the relative benefits of conventional vs. variable ventilation. This will likely require a multi-center format.

Project 2: We will establish a pre-clinical program conducted in laboratory mice with the objective of developing cell-based treatments for ALI.

The long-term goal of this project is to develop an autologous cell-based therapy to reconstitute the injured lung epithelium. A key element of this work is to evaluate and identify the optimal exogenous progenitor cell population with lung epithelial reparative properties. Since the start of this project, we have expanded our aims to include a newly discovered type of pluripotent stem cell, termed iPS cells, in addition to the originally proposed studies utilizing embryonic stem (ES) cells.

The recent discovery that pluripotent cells similar to embryonic stem (ES) cells can be generated by reprogramming fibroblasts via retroviral transfer of four transcription factors provides unprecedented opportunities to advance regenerative medicine. Like ES cells, the broad differentiative repertoire of these so called 'induced pluripotent stem (iPS) cells' suggests their use in treating patients suffering from degenerative diseases or injuries, including those involving the lung epithelium. In contrast to ES cells, iPS cells are genetically identical to the individual from whom they are derived, raising the prospect of utilizing iPS cells for autologous cell based therapies without risk of rejection. Significant obstacles must be surmounted, however, before iPS cells can be employed for lung epithelial reconstitution. First, as with ES cells, methods must be developed for the efficient in vitro derivation of lung epithelial progenitors from iPS cells, and subsequently for their engraftment in lung tissue. Second, the risk of tumorigenesis from transplanted iPS cells will need to be minimized before clinical use can be considered.

To date, our work developing tools to efficiently derive iPS cells has resulted in the engineering of a single lentiviral 'stem cell cassette' vector (1) able to efficiently reprogram fibroblasts into iPS cells that display the in vivo capacity to form a variety of endoderm-derived epithelia, including lung. In addition, in endoderm-promoting culture conditions, we show induction of putative endodermal marker genes in multiple iPS cell lines. Definitive endoderm is the embryonic precursor to lung epithelial progenitors and derivation of this germ layer in culture is a prerequisite for the efficient derivation of lung epithelial progeny. For this proposal we have derived iPS and ES cell lines that express reporter genes under the control of promoters active in the earliest stages of endodermal and lung epithelial development. This tool allows for the rapid and efficient evaluation of cells for lung regenerative potential.

To date, we have demonstrated that ES cells can be induced to sequentially recapitulate developmental milestones as they differentiate through a definitive endoderm stage into cells expressing markers of liver, pancreas, or lung lineages. We propose to adapt this approach to generate and purify similar derivatives from iPS cells.

For the remaining project period, we hypothesize that iPS cells possess endodermal and lung epithelial differentiative capacity equivalent to ES cells. We further hypothesize that very early (Ttf1+) lung epithelial progenitors can be derived from iPS

cells by ex vivo recapitulation of normal developmental milestones including definitive endoderm induction followed by specification of lung epithelial progenitors. We

propose that, once purified and characterized, these iPS-derived progenitors can be transplanted as an autologous cell-based therapy designed to reconstitute injured lung epithelium in bleomcyin treated mice. To test these hypotheses we are undertaking experiments involving the instillation of both ES-derived as well as iPS-derived endodermal and lung progenitors into mouse injury models as proposed in our original specific aims. We believe the inclusion of iPS cells in the project is a significant and exciting expansion of our originally proposed aims. We will, however, continue test cell populations cited in the parent proposal.

Key Research Accomplishments

- Study Coordinator hired
- Staff education regarding variable ventilation study underway
- Institutional Review Board approval of Phase I variable ventilation study
- FDA Pre-IDE completed; final IDE application awaiting final engineering adjustments and ventilator quality testing results
- Mouse iPS cells generated in our laboratory
- Development of iPS and embryonic stem cell lines with lung specific reporters
- Development of stem cell cassette vector for efficient generation of iPS cells

Reportable Outcomes

1) Establishment of iPS cell line that express lung epithelial specific reporters

2) Development of lentiviral vectors that enhance iPS cell derivation

3) Publications:

1. Kotton, D, and Fine, A. Lung Stem Cells. Cell, Tissue Research. 331:145-156, 2008.
2. Fine, A. Breathing Life into the Lung Stem Cell Field. Cell Stem Cell 4:468-469, 2008.
3. Sommer, CA, Stadtfield, M, Murphy, GJ, Hochedlinger, K, Kotton, DN, and Mosttoslavsky, G. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. Stem Cell. 27:543-549, 2009.

Conclusion

We have made considerable progress in both Projects of the original parent grant. For Project 1, we have successfully obtained IRB approval and are in the process of submitting an IND application to begin a Phase I study of variable ventilation. Software to support this endeavor has been developed and key personnel have been hired and are being trained. In Project 2, we have continued to develop potential sources of cell progenitor populations that can be utilized for reconstituting a damaged alveolar epithelial surface. One novel and very important extension of this has been the inclusion of iPS cells in these studies. For a variety of reasons these may prove to be a more valuable reagent for lung regeneration. This includes a capacity to generate cells that are genetically identical to the host that will receive them.

References

1. Sommer, CA, Stadtfield, M, Murphy, GJ, Hochedlinger, K, Kotton, DN, and Mosttoslavsky, G. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. Stem Cell. 27:543-549, 2009.

Title of Study: VARIABLE VENTILATION IN ACUTE LUNG INJURY
Protocol Number: H-27864
RE: New Protocol
Review Type: Full Board
Action: Approved
Date of Action: 4/9/2009
Date of Expiration: 4/8/2010
Funding Source:
Government: Department of Defense Award# W81XWH-08-1-0148 BUMC Source #057-298-5880-8: we will forward grant to IRB, it is too large to attach in INSPIR
Dear GEORGE O'CONNOR, MD:
The Institutional Review Board (IRB) has reviewed the above referenced protocol and has determined that it meets the requirements set forth by the IRB and is hereby approved. This protocol is valid through the date indicated above.
Approved pending submission of the IDE from the FDA.
This study is approved to use consent via Legally Authorized Representative.
Revisions have been reviewed and approved as of 6/26/2009.
The study may not continue after the approval period without additional IRB review and approval for continuation. You will receive an email renewal reminder notice prior to study expiration; however, it is your responsibility to assure that this study is not conducted beyond the expiration date.
Please be aware that only IRB-approved informed consent forms may be used when informed consent is required. Only consent forms validated with current approval dates (either generated by the INSPIR system or by a manual stamp by the IRB office) may be used. Manually stamped consent forms may be found under External Attachments in INSPIR.
Any changes to the protocol or informed consent must be reviewed and approved prior to implementation unless the change is necessary for the safety of subjects. In addition, you must report to the IRB unanticipated problems involving risk to subjects or others according to the process posted on the IRB website. The IRB must be informed of any new or significant information that might impact a research participant's safety or willingness to continue in your study.
Investigators are required to ensure that all HIPAA requirements have been met prior to initiating this study. Once approved, validated HIPAA forms may be found within

INSPIR as External Attachments.
It is the responsibility of the PI to ensure that all required institutional approvals have been obtained prior to initiating any research activities.
Please note that the IRB is no longer stamping attachments, subject letters, recruitment materials, etc. These documents are each associated with this approved version of the protocol. They can be found by going to Letters/Protocol History in

INSPIR and clicking on the highlighted (linked) word "Approved" and then clicking on the paperclip icon in the upper left corner. *This does NOT apply to consent

forms, which must be validated.
Sincerely yours,
JAMES FELDMAN
IRB Chair

I090104 – Boston University – Variable Ventilation Program for Nellcor Puritan-Bennett 840 Ventilator

Please find below our informal comments on your proposed device performance strategy and study design.

• Is there additional information that the FDA would require on the device specifications or device performance verification strategy?

The following additional information is recommended:

1. A detailed device description, including all components (software and interface to the ventilator) with the 510(k) number for all pre-market cleared devices should be provided.
2. Performance test validation reports with test methods, pass/fail criteria, results, and conclusion should be provided. For instance, what are the specifications and accuracies of the VV mode? Performance tests of these specifications should be done with a test lung.
3. More detailed pre-clinical study reports and any prior investigations should be provided.
4. Complete software documentation should be provided in accordance to "Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices" available at http://www.fda.gov/cdrh/ode/guidance/337.pdf and "Guidance for Off-the-Shelf Software Use in Medical Devices" available at http://www.fda.gov/cdrh/ode/guidance/585.pdf.
5. It is unclear what the "Nellcor Puritan-Bennett Performance Test System 2000" entails and how it compares to a pre-market cleared device. A description of this system should be provided.
6. A more detailed description of the VV mode/algorithm should be provided to define the operational principles of the mode and to gain a better understanding of the performance of the mode. For example, what values does the user set and what does the VV mode deliver in return? How much variable pressure (or volume) around a set mean does VV mode deliver/achieve?
7. A waveform comparison test of VV to ARDSNet using test lung should be provided.
8. Electrical safety and electromagnetic compatibility tests in accordance to IEC 60601-1 and IEC 60601-1-2 should be provided.
9. Risk analysis for the proposed changes to the device should be provided.

• Are there concerns about the proposed study design?

The following are concerns and questions about the proposed study design:

1. Full inclusion/exclusion criteria including the definition of ARDS/ALI recommended by American-European Consensus should be provided.
2. A statistical analysis plan including more details of the justification of study size

should be provided.
3. A detailed listing of all serum parameters to be evaluated and justification of the markers as indices of acute lung injury should be provided.
4. Details on how the variable ventilation settings will be chosen should be

provided. For example, will adjustments to the initial ventilator settings follow a protocol or algorithm based upon the patient's clinical status?
5. If supplemental oxygen levels may need to be adjusted during the trial period, the adjustments should be subject to a protocol and accounted for in the analysis.
6. Patients may enter with varying times on mechanical ventilation. Will there be an acceptable range of prior mechanical ventilation in the inclusion or exclusion criteria?
7. Differences in FiO2 may influence the propensity to lung injury. Will baseline oxygen requirements be considered in evaluating the results?
8. Fluid management strategies may influence outcomes in ARDS/ALI as shown by the FACTT trial. Will fluid management remain stable during the trial? Will fluid management changes be subject to protocol?
9. The first aim of the study involves tolerability of the mode. How will toleration of the mode be assessed?
10. Even though the study is a feasibility trial, it would be useful to generate success criteria with respect to the major aims of the study: safety, tolerability, oxygenation, mechanics, and lung injury.
11. There may be a delay in the effect of each mode based on "wash-out" of the effects of the prior mode. While the cross-over design with randomization of which mode is tested first should help with this problem, did the size calculation account for potential diminutions in effect based on prior ventilation modes?
12. The third aim of this study is to verify that variable ventilation results in lung mechanic improvement. How will the improvement be quantified and what are the success criteria?
13. It is unclear if suctioning will be allowed during the study period. Critically ill patients often require increases in the FiO2 during suctioning. Will adjustments in FiO2 during and after suctioning be tracked?
14. You stated that arterial blood gas samples to measure the pH, PO2, PCO2, and O2 saturation will be performed 15 minutes prior to the start of the study and 15 minutes prior to the end of each tested ventilator modes. Will arterial blood gases measurements be repeated once the subject reaches a steady state on the ventilator mode to determine the subject's tolerance to the mode and aid in the overall assessment of the subject? Will arterial blood gases be repeated if any changes to the initial ventilator settings are needed?
15. What conditions are anticipated that could result in inaccurate ETCO2 readings? What measures will be in place should ETCO2 readings become unattainable? What measures are in place should the pulse oximeter readings become unattainable?
16. Will all arterial blood gas sampling be analyzed by the same lab to eliminate variation that may occur in using different analyzers (e.g., hospital central lab, iSTAT analyzers, and etc.)?
17. Will prone positioning be excluded during the study period, since prone

positioning is commonly used in ALI/ARDS patients to improve the PaO2?
18. Will patient transport (e.g., CT scanning, MRI) be excluded during the study period as transporting a mechanically ventilated patient may require interruption from the ventilator mode?

• Are there concerns about the proposed study safety monitoring plan?
None at this time.

Additional comments and questions:
- Lung mechanic improvement should be assessed for both static and dynamic conditions.
- If ventilatory parameters are modified, how will they be optimized for ALI and ARDS patients?
- You stated that "variable ventilation produces, on average, greater alveolar recruitment and hence higher lung volumes." How was this (or will this be) demonstrated?
    - Is the device intended for use in magnetic resonance environments?